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Variation in the promoter of the human hormone sensitive lipase gene shows gender specific effects on insulin and lipid levels: results from the Ely study

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Abstract

We previously identified a hormone sensitive lipase (*HSL*) promoter variant, −60C > G, which in vitro exhibits 40% reduced promoter activity. In this study we examined the effect of the −60C > G on glycemic and lipid measures in the population based Ely study of metabolic function and insulin resistance in 218 middle-aged men and 276 middle-aged women. Adipose tissue HSL is the rate-limiting step in triglyceride lipolysis, generating free fatty acids for energy utilization. HSL is also expressed in pancreatic β-cells where its activity therefore may affect insulin secretion. In the women, carriers of the *HSL* −60G allele had significantly lower fasting insulin levels ($P = 0.0005$) and a lower total area under the curve for insulin during the oral glucose tolerance test ($P = 0.005$). There was no demonstrable association in men with these measures of insulin sensitivity but carriers of the −60G allele had significantly lower fasting non-esterified fatty acid (NEFA) levels ($P = 0.025$) and higher low density lipoprotein cholesterol levels ($P = 0.02$) than men who were non-carriers. This study provides additional evidence for a role for HSL in the development of insulin resistance, from which carriers of the −60G allele, associated here with markers of insulin sensitivity in women, and with lower NEFA levels in men, might be protected.

Keywords: Hormone sensitive lipase; Insulin resistance; Fasting insulin levels; Genetic polymorphism

1. Introduction

Dyslipidemia and insulin resistance are both features of type 2 diabetes, with the increase in free fatty acid (FFA) flux stimulating very low density lipoprotein production [1] and glucose stimulated insulin secretion (GSIS) [2], being a unifying factor. Hormone sensitive lipase (HSL), the rate-limiting enzyme in the intracellular hydrolysis of triglycerides (Tg), is a major regulator of free fatty acid release and therefore plays a central role in the control of FFA flux. HSL activity is tightly controlled in the post-absorptive state by catecholamine activation via cAMP-dependent phosphorylation and, in the fed state, by the dephosphorylation by insulin (reviewed in [3]). Thus HSL is post-translationally activated to provide

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FFAs when energy intake is low, and deactivated when exogenous sources of energy are available. As a key enzyme in FFA homeostasis, HSL is a candidate gene for insulin resistance.

The coding region of the *HSL* gene is remarkably invariant [4,5] and the only reported polymorphic site, resulting in the R309C substitution, was identified in Japanese [6] and Chinese samples but not in Caucasians [5]. In a small study in Japanese type 2 diabetics and healthy controls, the C309 allele was shown to be associated with higher plasma total cholesterol but not plasma Tg or obesity [6]. In addition, two dinucleotide repeat minisatellites have been used in population and transmission disequilibrium studies to examine the association of genetic variation in *HSL* with central obesity and type 2 diabetes. Magre et al. found an association between the intron 6 repeat with central obesity and type 2 diabetes [7], while Klannemark et al., using the intron 7 repeat, reported a significant difference in the distribution of the alleles in centrally obese and type 2 diabetic patients compared to controls, as well as transmission disequilibrium in the type 2 diabetic families [4]. These frequency studies implicate HSL in type 2 diabetes, without identifying the functional change.

We have reported a C > G change at position −60 in the HSL promoter with the rare allele being associated with a 40% reduction in promoter activity in vitro, which suggests a decrease in *HSL* gene expression [5]. This suggested that variation in the transcriptional control of *HSL*, in addition to the post-translational regulation of HSL activity, may also take place. We have now examined the effect of the −60C > G variant on repeat lipid and glycemic measures taken 4.4 mean years apart, in 494 middle aged men and women participating in the prospective Ely study of metabolic precursors of type 2 diabetes [8].

2. Materials and methods

2.1. Sample population and biochemical measures

The Ely study is a prospective population-based study of the etiology and pathogenesis of insulin resistance and related disorders which has been described previously [8]. Briefly, subjects who were not known to have type 2 diabetes were randomly recruited from a sampling frame consisting of all those aged 40–65 years in the single GP practice in Ely, Cambridgeshire in April 1990. Overt diabetics were excluded from the study. 1122 subjects (74% response rate) agreed to participate and attended the local surgery after a 10 h fast for a clinical examination which included a standard 75 g OGTT (blood drawn at 0, 30 and 120 min), anthropometric measurements and a dietary and medical questionnaire. Biochemical measures were taken as previously reported [8,9]. Plasma non-esterified fatty acids (NEFA) were determined enzymatically based on acyl-CoA synthetase activity (Boehringer Mannheim, Lewes, Sussex, UK) [9]. The cohort was then remeasured 4.4 years later, and 937 of the 1071 individuals who were non-diabetic at the first sampling underwent the same evaluation as in 1990–1992. The sample of individuals initially considered for this analysis was a random subgroup of 534 individuals who had complete data at both phases of the Ely study and on whom a DNA sample was available. In the analysis we restricted attention to the 494 individuals who were non-diabetic and who were not receiving pharmaceutical therapy for dyslipidemia at any stage during this 4.4 year period.

2.2. Polymorphism detection

Detection of the *HSL* −60C > G polymorphism by PCR and enzyme digestion has been previously reported [5].

2.3. Statistical analysis

The data presented utilized both measurements during this study with an average of 4.4 years between repeated measures. We hypothesized that a gene effect would determine the 'usual' level of insulin sensitivity, which is a latent or unmeasurable variable. However, it can be estimated by repeated measurement. In this study, therefore, the primary outcome variable was the repeated measurement of fasting insulin and area under the curve (AUC) for insulin following an oral glucose tolerance test. We did not hypothesize that the HSL polymorphism would affect the rate of change of these variables over time. Such an analysis would probably require longer follow-up in a larger population with more

Table 1
The unadjusted means by using average values for variables that vary over time, in the men and women

	Men				Women			
	WT (193 CC)		Rare allele carriers (23C/G+2GG)		WT (252 CC)		Rare allele carriers (24C/G)	
	mean	S.E. or 95% CI	mean	S.E. or 95% CI	mean	S.E. or 95% CI	mean	S.E. or 95% CI
Age (years)	56.6	0.56	55.1	1.54	55.0	0.47	54.4	1.52
Body mass index (kg/m^2)	26.28	0.20	26.3	0.60	26.0	0.30	25.6	0.89
Waist to hip ratio	0.934	0.004	0.920	0.01	0.785	0.004	0.765	0.01
Fasting insulin (pmol/l)[a]	42.69	(39.60, 46.02)	42.40	(34.35, 52.34)	42.46	(39.75, 45.36)	30.93	(27.11, 35.30)[c]
30 min insulin increment[a]	27.00	(24.54, 29.71)	27.15	(21.47, 34.34)	31.80	(29.43,34.36)	27.28	(21.82, 34.12)
Insulin AUC (pmol·h/l)[a]	481.5	(444.9, 521.0)	491.7	(396.4, 609.9)	504.3	(473.8, 536.9)	408.1	(340.2, 489.6)[b]
Fasting NEFA (mmol/l)	0.44	0.013	0.35	0.026[b]	0.54	0.014	0.53	0.036
NEFA 120 min (mmol/l)	0.086	0.003	0.084	0.010	0.073	0.002	0.065	0.006
Cholesterol (mmol/l)	6.19	0.07	6.52	0.16	6.43	0.07	6.29	0.24
LDL cholesterol (mmol/l)	4.23	0.06	4.59	0.16	4.284	0.07	4.12	0.20
Triglycerides (mmol·h/l)[a]	1.33	(1.25, 1.41)	1.54	(1.20, 1.96)	1.15	(1.08, 1.21)	1.15	(0.99, 1.34)

Mean (S.E.) or [a]geometric mean (95% CI); [b]$P < 0.05$; [c]$P < 0.01$.

precise measurement of insulin sensitivity. This study was not powered to examine the genotypic effects on type 2 diabetes.

One way to display the repeated measures is simply to average them over the two repeats within an individual. Table 1 shows the means of these averaged data by carrier/non-carrier status stratified by sex. Comparison between carriers and non-carriers was by *t*-test. Traits that were not normally distributed, e.g. plasma insulin, Tg and related measures, were normalized after log transformation and geometric means used as the measure of central tendency.

An alternative way of describing the association between the genetic polymorphism and the repeated measures of the outcome variables (fasting insulin and AUC insulin) used a mixed model (SAS V.8, SAS Institute, Cary, NC, USA). For this a series of multivariate regression models were undertaken to describe the effects of genotype on quantitative phenotypic outcome using the mixed model approach. This allows time varying (e.g. body mass

Table 2
β coefficients from the regression models for the effect of HSL −60C > G genotype for fasting insulin, 30 min insulin and AUC for insulin measures after the OGTT and for fasting triglycerides, cholesterol, NEFA and LDL cholesterol (baseline group *HSL* −60CC)

	Men ($n = 218$)			Women ($n = 276$)		
	β coefficient	S.E.	*P*-value	β coefficient	S.E.	*P*-value
BMI$^+$ (kg/m^2)	0.298	0.45	0.51	−0.266	0.735	0.72
Waist to hip ratio[a]	−0.007	0.01	0.49	−0.020	0.01	0.05
Log fasting insulin[b] (pmol/l)	−0.034	0.09	0.70	−0.281	0.080	0.0005
Log 30 min insulin[b] (pmol/l)	−0.131	0.11	0.22	−0.179	0.11	0.1025
Log insulin AUC[b] (pmol/l/h)	−0.045	0.09	0.60	−0.210	0.07	0.0054
Fasting NEFA[b] (mmol/l)	−0.073	0.03	0.0251	0.014	0.036	0.70
120 min NEFA[b] (mmol/l)	−0.003	0.01	0.80	−0.007	0.007	0.30
Cholesterol[b] (mmol/l)	0.313	0.16	0.051	−0.118	0.18	0.50
LDL cholesterol[b] (mmol/l)	0.351	0.15	0.020	−0.15	0.16	0.33
Log triglycerides[b] (mmol/l)	0.078	0.07	0.28	0.011	0.07	0.87

[a]Adjusted for age, BMI and smoking status.
[b]Adjusted for age and smoking status.

index) and fixed covariates (e.g. sex) to be considered in the same model as independent variables and allows repeat measures of quantitative outcome to be used as the dependent variable.

3. Results

As presented in Table 1, among the women there were 252 −60CC homozygotes and 24 −60C/G heterozygotes. There were no women homozygous for the rare allele. In the men there were 193 −60CC homozygotes, 23 −60C/G heterozygotes and two rare allele −60G/G homozygous individuals. Genotypic proportions were in Hardy–Weinberg equilibrium with the frequency of the rare −60G allele being 0.06. Table 1 shows the crude data comparing carriers and non-carriers for anthropometric and biochemical parameters. In the men the two individuals who were homozygous for the rare allele were combined with heterozygotes in the analyses. However, when these two men were removed from subsequent analyses there was no difference in the outcome (data not shown). In women, fasting insulin ($P < 0.01$) and

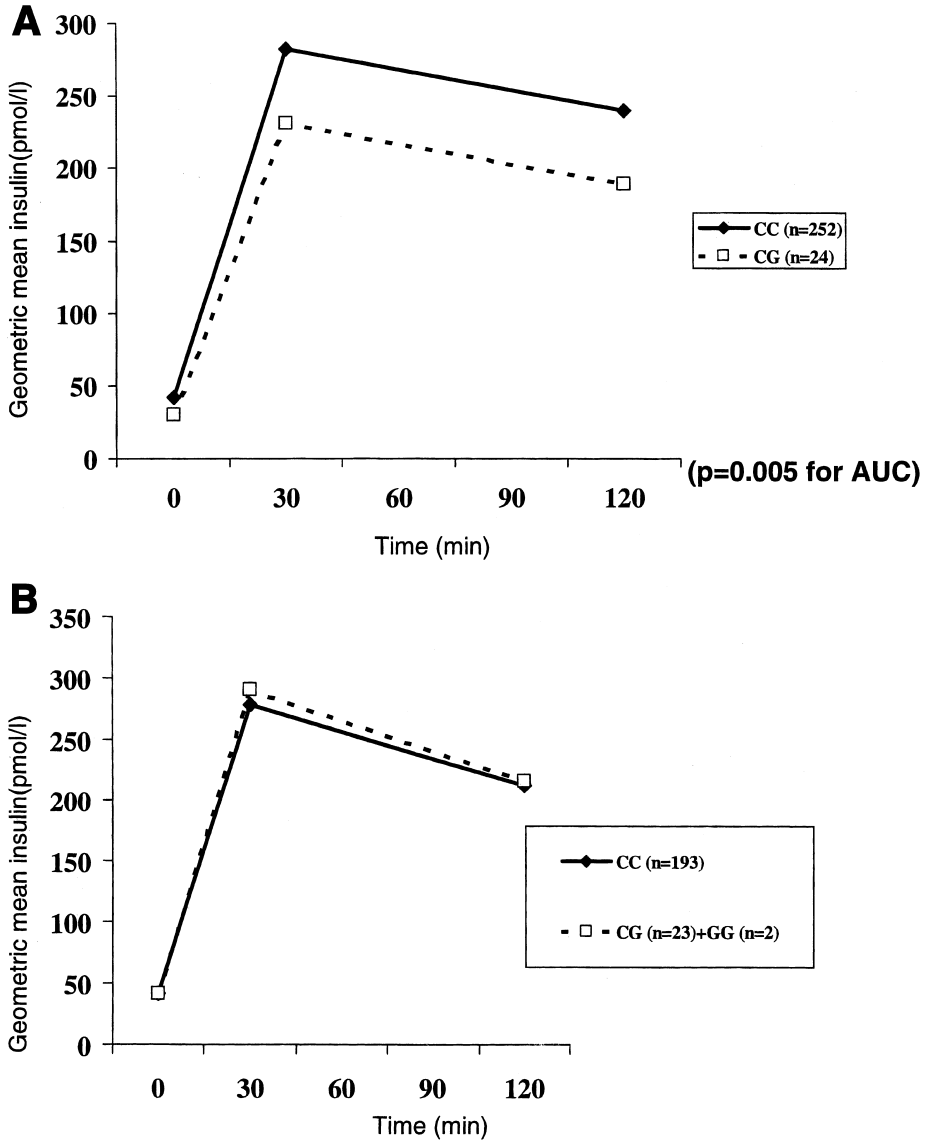

Fig. 1. Insulin response after the OGTT in women (A) and men (B) participating in the Ely study, according to *HSL* −60C > G genotype.

AUC insulin concentration ($P < 0.05$) were significantly lower in heterozygous carriers of the −60G allele than in non-carriers. No such associations were demonstrable in men. In men, however, the fasting NEFA concentration was significantly lower in carriers (−60C/G+GG) than in non-carriers (−60CC) ($P < 0.05$). These crude data do not take into account potential confounding factors and therefore we undertook the more complicated mixed model regression analysis in order to allow for the inclusion of time varying covariates such as obesity and cigarette smoking. Results from the regression analyses are presented in Table 2. In the women ($n = 279$), compared to those homozygous for the common −60C allele (−60 CC), carriers of the −60G allele (−60 CG) had lower fasting insulin ($P = 0.0005$), and an enhanced response to an oral glucose tolerance test as reflected in the lower area under the curve for insulin ($P = 0.005$). The adjusted mean insulin concentration at the different time points during the oral glucose tolerance test stratified by genotype are shown in Fig. 1. In addition, the waist–hip ratio in those women who were carriers of the −60G was lower than in the −60C homozygotes, but the difference was only of borderline significance ($P = 0.05$). Although the same trend of association was observed in men ($n = 218$), the differences between genotype were not statistically different. However, those men who were carriers of the −60C > G variant had significantly lower fasting NEFA levels ($P = 0.025$). Low density lipoprotein (LDL) cholesterol levels were higher in men who were −60G carriers compared to CC homozygotes ($P = 0.02$) (Table 2), but these differences were not seen in women.

4. Discussion

This study demonstrates that women who are homozygous for the common *HSL* −60C allele tend to have higher fasting insulin concentrations and an exaggerated insulin response to a glucose challenge, both markers for insulin resistance. As insulin resistance is a metabolic precursor of type 2 diabetes, these data suggest that the rare −60G allele may be protective. Although the direction of association was similar in men, the size of the effect was smaller and was not statistically significant. These results are in agreement with those of Pihlajamaki et al. [10], who recently reported an association between the *HSL* −60C > G and insulin stimulated whole body glucose uptake, suggesting that the −60G allele was protective of insulin resistance in men. In our study, however, in men the rare allele of the *HSL* −60C > G was associated with lowered fasting NEFA levels, which have been shown previously to be associated with other features of the insulin resistance syndrome, and which differ markedly between the sexes [9].

The control of *HSL* gene expression is under at least two different promoters. The testis-specific promoter controls *HSL* expression in the seminiferous tubules and produces a transcript that has the addition of a testis-specific exon [11]. Expression of *HSL* in all other tissues [12] appears to be directed by the adipose tissue promoter. Post-translationally controlled by catecholamines and insulin the expression of *HSL* in adipose tissue varies according to the type of adipose tissue, and is dependent upon the expression of α- and β-adrenoceptors [13]. Thus HSL activity differs in subcutaneous fat compared to visceral or ormental fat [14,15]. In smooth and cardiac muscle HSL lipolysis of triglycerides provides FFA energy via β-oxidation [16,17].

The balance between the usage of glucose and FFA as energy sources (the Randle hypothesis [18]) highlights the fact that raised FFA can impair glucose metabolism and alter insulin sensitivity [19,20]. Thus while insulin is involved in the control of HSL activity, this study suggests the intriguing possibility that HSL is itself determining insulin levels. Fatty acids enhance GSIS within the pancreatic β-cells (reviewed in [21]). It was recently reported that HSL is expressed and active in the β-cells [22] and this raises the possibility that HSL may mobilize FFA from intracellular Tg stores in the β-cells. While on the one hand HSL activity is controlled by insulin, it may itself contribute to the control of insulin secretion by generating long-chain FFA that participate in the signaling process in GSIS in addition to the FFA flux from adipose tissue activity [2]. However, to date these is no evidence that the *HSL* adipose tissue promoter is active in the pancreatic β-cells. Thus this interpretation is speculative and needs to be ratified experimentally.

Whether the −60C > G variant is indeed the functional change leading to these associations or is in allelic association with a yet unidentified functional site, needs further study. We have evidence (unpublished data, Talmud and Palmen) that there is no allelic association between this promoter variant and the intron 6 or intron 7 CA repeats. Since both variable repeat sequences have been found to be associated with obesity and type 2 diabetes, this strengthens the argument that variation in the *HSL* gene, as identified by these three sites, is involved in the pathophysiology of insulin resistance and thus a precursor to type 2 diabetes. Results from our study suggest that the major impact of genetic variation in *HSL* is indeed on insulin metabolism and not on total body fat.

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